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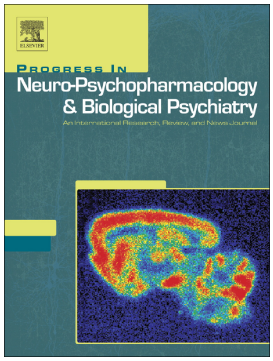

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NEURAL ACTIVATION DURING COGNITIVE REAPPRAISAL IN GIRLS AT HIGH RISK FOR DEPRESSION

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ABSTRACT

Objective: Although emotion dysregulation, one of the core features of depression, has long been thought to be a vulnerability factor for major depressive disorder (MDD), surprisingly few functional magnetic resonance imaging (fMRI) studies have investigated neural correlates of emotion regulation strategies in unaffected high risk individuals.

Method: Sixteen high risk (RSK) young women and fifteen matched low risk controls (CTL) were scanned using fMRI while performing an emotion regulation task. During this task, participants were instructed to reappraise their negative emotions elicited by International Affective Picture System images (IAPS). In addition, Difficulties in Emotion Regulation Strategies Scale (DERS) was used to assess participants' emotion dysregulation levels.

Results: Both RSK and CTL individuals show increased amygdala activation in response to negative emotional stimuli, however no difference was found between groups in using cognitive reappraisal strategies and functions of brain regions implicated in cognitive reappraisal. Interestingly, our psychometric test results indicate that high risk individuals are characterised by lower perceived emotional clarity (EC).

Conclusion: Results of the current study suggest depression vulnerability may not be linked to the effectiveness of cognitive reappraisal. Alternatively, lower EC may be a vulnerability factor for depression.

Key words: Depression; High risk; fMRI; Emotion regulation; Reappraisal

Highlights:

- Emotion dysregulation is one of the core features of depression.
- It is not clear whether emotion dysregulation precedes long before the first depressive episode in high risk individuals.
- In this study, individuals at high risk for depression use efficiently cognitive emotion regulation strategies to ameliorate their induced negative emotions.
- Compared to healty controls, unaffected high risk individuals show significant difficulties in understanding, identifying, and differentiating their own emotions (low emotional clarity).
- Low emotional clarity may be a new endophenotype for depression.

Introduction

Major Depressive Disorder (MDD) is among the most prevalent mental disorders that cause marked impairment in social and occupational functioning, and quality of life (APA, 2013). It is estimated that 150 million people worldwide are affected with MDD at any moment in time, and one in every five women and one in every eight men experience a MDD episode during their lifetime (Kessler et al. , 1994, Wang et al. , 2007). In addition, depression is frequently comorbid with other psychiatric and medical disorders (APA, 2013). Most importantly, 15% of depressed patients eventually die by suicide (Simon and VonKorff, 1998).

Although effective treatments are available, it has been estimated that, even under optimal conditions, current treatments (e.g. medications and psychotherapy) can reduce only about one third of the disease burden associated with MDD (Andrews and Wilkinson, 2002, Chisholm et al. , 2004). A plausible way of reducing disease burden of MDD might be reducing the number of new cases. This can be done by prevention rather than treatment, as recent studies have consistently shown that intervention in high risk individuals can reduce later transition to depression (van Zoonen et al. , 2014). Therefore, it is important to identify and use prevention strategies for individuals who are at high risk of depression before they have experienced a depressive episode.

Although there are some well known vulnerability factors for the development of depression such as age, gender, adverse childhood experiences, neuroticism, and family history of MDD not all people with these risk factors will go on to develop a depressive episode. The challenge is to predict those individuals most likely to make this transition, because there are currently no tests or biological markers that can assist in making early diagnosis of this disorder. There is thus a pressing need to identify neurobiological markers that can identify those high risk subjects who are most likely to become depressive, so that clinical resources could be focused on this subgroup. Insight in this process may aid to understand the neural

underpinnings of the onset of MDD, which is vital to improve treatment and prevention strategies.

Emotion dysregulation is one of the core features of MDD (Gotlib and Joormann, 2010) . In addition, previous research has suggested that MDD may result from the inability to down regulate negative emotions through cognitive emotion regulation strategies such as reappraisal, acceptance, or problem-solving (Billings and Moos, 1981, D'Zurilla et al. , 1998, Nolen-Hoeksema, 2012) . Among them cognitive reappraisal is by far the most studied emotion regulation strategy through functional neuroimaging methods in both healthy individuals and depressed patients. Therefore, neural correlates of reappraisal in unaffected high risk individuals may provide more insight in biological risk markers of MDD. However, no study to date has examined neural correlates of cognitive reappraisal in unaffected high risk individuals relative to low risk controls.

During cognitive reappraisal, one attempts to reinterpret an emotion-eliciting situation in a way that alters its meaning and reduces its emotional impact (Gross, 2002). Available data suggests that the beneficial effects of reappraisal are provided through the interactions between the amygdala and regions of the prefrontal cortex (Buhle et al. , 2014). The dorsolateral prefrontal cortex (DLPFC), which relates to executive control of cognitive functions, is among the most consistently activated prefrontal cortex region in neuroimaging studies of reappraisal (Kalisch, 2009, Ochsner and Gross, 2005). The other important brain region in cognitive emotion regulation, the amygdala, is involved in the evaluation of and response to emotional stimuli (LeDoux, 2000, Zald, 2003).

In healthy controls, investigators have found reasonably consistent associations between exposure to negative stimuli and increased amygdala activation (Costafreda et al. , 2008). This increased amygdala activation elicited by exposure to negative stimuli is significantly reduced during reappraisal. Modulation of amygdala activation is accomplished by the

increased activation of DLPFC (Ochsner and Gross, 2008). In other words, the functional coupling between DLPFC and amygdala may reflect the effectiveness of reappraisal.

With respect to depression, the main brain regions implicated in reappraisal, amygdala and DLPFC, appear to be dysfunctional. It has been reported that compared to healthy controls, depressed patients show greater amygdala activation when exposure to negative emotional stimuli (Hamilton and Gotlib, 2008). Studies of both resting-state brain perfusion and glucose metabolism have consistently revealed lower levels of DLPFC activation in depressed patients than healthy controls (Biver et al. , 1994). In addition, neuroimaging studies of cognitive reappraisal have reported reduced DLPFC activation, decreased capacity to reduce amygdala activation, and reduced DLPFC-amygdala coupling in response to negative stimuli in depressed patients (Erk et al. , 2010, Greening et al. , 2014).

In recent years, researchers have begun to explore the extent to which these abnormalities are present in high risk individuals. Results of these studies are intriguing. For example similar to depressed patients, high risk individuals show greater amygdala activation in response to negative stimuli than the controls (Chan et al. , 2009, Levesque et al. , 2011, Monk et al. , 2008, Wolfensberger et al. , 2008). In addition, it has also been reported that relative to controls, high risk individuals have diminished activation of the DLPFC in response to the presentation of fearful faces (Mannie et al. , 2011) and attempt to ameliorate sad mood by recalling positive memories (Joormann et al. , 2012). Although these findings suggest that altered limbic and prefrontal functioning precedes long before a depressive episode and serves as a neurobiological marker for depression vulnerability, it is clear that empirical evidence for an emotion dysregulation view of depression vulnerability is still limited and further investigation is warranted.

The aim of this present study was to extend our understanding of neurobiological markers for depression vulnerability. Based on the literature described above, we conducted an fMRI

study of cognitive reappraisal and hypothesized that high risk individuals, relative to low risk controls, would report more difficulties in using reappraisal strategies and show greater activation in the amygdala in response to negative stimuli, and less activation in prefrontal cortex regions during ameliorating of negative affect.

Young daughters of mothers with recurrent MDD were identified as the high risk group of our study. The rationale behind this choice comes from following studies: 1) MDD generally peaks in the early adulthood period and females experience depression 1.5- to 3-fold higher rates than men (Kessler et al. , 2003); 2) Offspring of parents with MDD face a three times greater risk for MDD than offspring without such a family history (Weissman et al. , 2006); 3) Maternal depression is particularly associated with depression in offspring by age 24 (Klein et al. , 2005); 4) Daughters of depressed mothers are more vulnerable to depression than sons (Davies and Windle, 1997). Taken together, young women with mothers suffering from recurrent MDD appear to be one of the most favourable high risk populations in order to investigate neurobiological markers for depression vulnerability.

MATERIAL AND METHODS

Participants

Forty two right-handed girls between the ages of 18 and 24 years with no past or current DMS-IV axis I disorder participated in the study. Twenty two girls had biological mothers suffering from recurrent MDD (high risk group [RSK]), and 20 girls had biological mothers with no history of any axis I disorder (low risk group/control [CTL]).

Eleven participants (6 RSK and 5 CTL) were subsequently excluded for high mood scores and/or excessive movement in the MRI scanner, leaving a total of 31 participants who completed the study.

High risk participants were recruited among daughters of female patients with recurrent MDD attending both Ege University School of Medicine's psychiatric outpatient clinic and other local psychiatric outpatient clinics in Izmir. Depressed patients were diagnosed by a consultant psychiatrist and did not have any psychiatric comorbidity. The healthy control subjects were volunteers via advertisements posted in numerous locations ççwithin the local community.

Participants eligibility for the study was evaluated by a psychiatrist using following self-and observer-rated scales: Structured clinical interviews for DSM-IV (SCID)-I (Spitzer et al. , 1992), Hamilton Depression Rating Scale for Depression (HDRS) (Hamilton, 1969), State-Trait Anxiety Inventory (STAI) (Spielberger et al. , 1983) . All participants scored within normal range (0-8) for healthy individuals on the HDRS. The mean scores of both groups were well below the clinical cut-off point (>8) for depression. In addition, the Difficulties in Emotion Regulation Scale (DERS) (Gratz and Roemer, 2004) was used to assess participants' emotion dysregulation levels. The DERS is a brief, 36-item self-report questionnaire designed to assess multiple aspects of emotional dysregulation. Higher scores suggest greater problems with emotion regulation.

Handedness was determined by the Edinburg Handedness Inventory (Oldfield, 1971). Written informed consent was obtained from all subjects subsequent to a detailed description of the

study. The study design, approved by the local ethics committee of the Ege University School of Medicine, was prepared in accordance with the ethical standards laid down in the Declaration of Helsinki. All participants were paid for their participation in the study.

The exclusion criteria of the study were as follows: left handed, current pregnancy, a current or past psychiatric or neurological disease, a previous or present head injury, a current medical disease influencing the central nervous system, inability to read and see stimuli presented on the screen, any contradictions to magnetic resonance imaging (i.e. metal in the body or claustrophobia).

Task

In order to test our hypotheses, we created an emotion regulation task adapted from an experimental procedure initially developed by Jackson et al. (Jackson et al. , 2000). The task consisted of 48 neutral and 48 negative images. All images were selected from the International Affective Picture System (IAPS) and balanced for valence and arousal (Lang et al. , 1993). Neutral images were primarily used to avoid habituation effects and are not a focus of the analyses presented here. To avoid habituation effects, negative and neutral images were pseudo-randomly intermixed, and images from the same category were presented no more than three times in a row. The task was programmed using Presentation® software (Neurobehavioral Systems, Albany, CA, USA). Images were presented via binocular fiber-optic goggles (Nordic Neurolab, Bergen, Norway) connected to the head coil using the same software.

Each trial was composed of five parts (Figure 1). First, a negative or neutral image was shown for 4 seconds with the written instruction "View" underneath the image. During this part participants were instructed to view and understand the content of the picture. Second, the image remained on the screen for an additional 4 seconds with an instruction either to "Attend" or "Reappraise" replacing the instruction to "View". The “Attend” instruction was

used for both negative and neutral images, indicating that the participant should maintain their attention to the image without changing their affective experience elicited by the image. The "Reappraise" instruction was used only for negative images, indicating that participant should reinterpret the image so that it no longer induced a negative response. Since long duration tasks may cause severe motion artefacts in fMRI analysis and the main focus of the current study was examining the neural correlates of reappraisal, the reappraise instruction was paired 30 times, and the attend instruction was paired 18 times for negative images. Third, the image disappeared and a black screen was shown for 4 seconds. Fourth, to evaluate the success of reappraisal, four rating words and corresponding numbers [not at all (1), slight (2), moderate (3), strong (4)] each with a 2 second duration were shown consecutively. Participants were instructed to make a key press with their right thumb using a fiber-optic one-button response system (Nordic Neurolab) located in their right hand, when they saw the appropriate word reflecting their current negative feeling. Responses were collected using Presentation software. Finally, the word "Relax" appeared in the center of the screen with a 2 second duration, serving as a short rest period until the next trial began.

Figure 1

Prescan Training

Participants received cognitive reappraisal training using a set of pictures immediately before entering the scanner. The pictures used in the training session were different from those used as stimuli for the fMRI measurements. The training was developed and administered by a clinical psychologist. During the training session, participants were taught how to generate

less negative interpretations of unpleasant scenarios in order to change their emotional response.

Neuroimaging Data Acquisition

Whole-brain functional and anatomical images were acquired using a 3.0 Tesla Siemens MAGNETOM Verio MRI scanner with Syngo software and 12-channel Head Matrix coil located at the department of Neuroradiology, Ege University School of Medicine. The MRI protocol consisted of the acquisition of a high-resolution three-dimensional T1-weighted structural scan, followed by the fMRI experiment. High-resolution T1-weighted MP-RAGE gradient-echo anatomical images were collected with 160×1 mm sagittal slices (TE: 2.21 ms., TR:1600 ms., TI:900 ms., FOV: 256×256 mm, image matrix resolution: 256×256). Functional images were acquired using a T2*-weighted gradient-echo echo planar imaging (EPI) pulse sequence with 23×4 mm slices, interleaved from bottom to top (interslice gap: 1 mm, TE: 30 ms., TR: 2000 ms., flip angle: 60^{o}, FOV: 192×192 mm, in-plane matrix resolution: 64×64, 1056 dynamic scans with 2 second duration). Head movement artefacts and scanning noise were restricted using head cushions and headphones within the scanner coil. The fMRI session always occurred from 16:00 p.m to 17:00 p.m. after the fMRI training session.

fMRI Data Analysis

Preprocessing

The functional imaging data preprocessing was completed using the Statistical Parametric Mapping software (SPM8) (http://www.fil.ion.ucl.ac.uk/spm/software/spm8/), implemented in Matlab (Mathworks, Natick, MA, USA). The first 11 scans were discarded to allow for T1

equilibration effects. The EPI images were then realigned to the first volume in order to correct for head movements. Realignment parameters were checked to discern any potential subjects with excessive head movement, defined as any displacement above 4 mm from the position of the first acquired fMRI volume in a session. Each participant's structural image was coregistered to the mean of the motion corrected functional images using 12 parameter affine transformation. The structural images were then normalized to MNI space and the same transformation was applied to the coregistered functional images and functional images were normalized to an MNI (Montreal Neurological Institute) EPI template with 2x2x2 mm voxel size. Finally, normalized images were smoothed using 8 mm full width, half maximum (FWHM) Gaussian Kernel.

STATISTICAL ANALYSIS

Statistical Analysis of fMRI Data

After preprocessing, statistical analysis for each individual subject was conducted using general linear model in SPM8 . At the first level, 5 task related regressors (View Negative, View Neutral, Attend Negative, Reappraise Negative, Rate) were modeled with a boxcar

function convolved with a hemodynamic response function. The six parameters of the participant's head movement (realignment parameters) were also included as regressors in the model, resulting in 10 regressors. After parameter estimation, the following two contrasts of interest were created: "View Negative" > "View Neutral", "Reappraise Negative" > "Attend Negative". The first contrast was used to assess the neural correlates of emotional response of participants to negative stimuli (negative emotion processing). The second contrast was used to assess the neural correlates of reappraisal. These contrasts were entered into a full factorial second level analysis model using two independent groups (RSK and CTL group) as factors. Age was entered in the model as covariate. The significance threshold was set to $p < 0.05$, family-wise error corrected for multiple comparisons across the whole brain, or for multiple comparisons within an anatomically defined region of interest (ROI) (amygdala, DLPFC) provided by the Wake Forest University PickAtlas (www.fmri.wfubmc.edu).

Statistical Analysis of Non-imaging Data

Due to the small sample size in each group, demographic and clinical characteristics for high- and low-risk participants were compared using Mann-Whitney U Test for continuous variables. Participants' self-reported emotion severity scores (in scanner) were evaluated via a 2 (Group: RSK, CTL) x 3 (Trial type: reappraise-negative, attend-negative, attend-neutral) repeated measures analysis of variance (ANOVA) to check whether RSK and CTL group differ in effectively using cognitive reappraisal strategies to regulate their emotions. The level of statistical significance was set at $p < 0.05$. All statistical analyses were performed using the Statistical Package for the Social Sciences (version 18.0, SPSS, Chicago, IL, USA).

RESULTS

Participant Characteristics

Demographic and clinical characteristics of the two participants groups are presented in Table 1. There were no significant group differences in age and duration of education. High-risk

participants reported significantly higher scores on STAI-S, STAI-T, and DERS-Clarity than did the low-risk participants.

Table 1

Behavioral Results

Self-reported emotion regulation during the fMRI task was evaluated via a 2 (Group: RSK, CTL) x 3 (Trial Type: reappraise-negative, attend-negative, attend-neutral) repeated measures ANOVA. There was a significant main effect of Trial Type, $F(2,58)=144.22$, $p<.001$. Follow-up paired t tests indicated more negative affect ratings on attend-negative trials than reappraise-negative trials, $t(30)=9.59$, $p<.001$, and more negative affect ratings on reappraise-negative trials than attend-neutral trials, $t(30)= 7.46$, $p<.001$. There was no main effect of

Group, F(1,29)= 0.38, p=.54, or Group Trial Type interaction, F(2,58)=0.88, p=.42. These results indicate that both groups reported successful emotion regulation, with no differences between groups.

Neuroimaging Results

Within-Group fMRI Contrasts

Inducing negative emotion

We contrasted View Negative scan with the View Neutral scan ["View Negative" > "View Neutral", (first contrast)] to identify brain regions involved in generation of negative emotions induced by negative pictures. Table 2 and 3 presents brain regions activated during this contrast in RSK and CTL group, respectively. While both groups show increased activation in

amygdala, prefrontal cortex, and temporal cortex, the CTL girls showed substantial activation in brainstem. The RSK girls also showed additional activation in inferior frontal gyrus, lingual gyrus, fusiform gyrus and precuneus.

Repairing negative emotion

We also contrasted Reappraise Negative scan with the Attend Negative scan ["Reappraise Negative" > "Attend Negative", (second contrast)] to identify brain regions associated with cognitive reappraisal. Both the CTL and RSK groups showed increased activation in prefrontal cortex, temporal cortex, cerebellum and precuneus. In addition, the RSK girls showed activation in cuneus, and caudate nucleus; in contrast, the CTL girls showed extensive additional activation in anterior cingulate cortex (BA 32) and supramarginal gyrus (Table 2 and 3).

Between-Group Comparisons

In contrast to our expectations, no difference was observed between groups for the contrast "View Negative" > "View Neutral" and "Reappraise Negative" > "Attend Negative". It is important to note that there was no significant difference between groups even when the threshold was lowered to an uncorrected $p<0.001$.

ROI Analysis

Based on our a priori hypotheses, ROI analyses were performed by extracting parameter estimates of blood oxygen level dependent (BOLD) signals from the bilateral amygdala and bilateral DLPFC. These anatomical regions were provided by the Wake Forest University PickAtlas (www.fmri.wfubmc.edu). DLPFC ROI's corresponded to Broadmann's areas 9 and 46. As expected, both groups exhibited increased bilateral amygdala activation in response to negative pictures (Figure 2) and increased DLPFC activation during using cognitive

reappraisal strategies. However, contrary to our hypothesis ROI analysis yielded no group difference in amygdala and DLPFC activations.

Figure 2

Correlation Analysis

We found no significant correlation neither between psychometric test scores and activation magnitudes of both amygdala and prefrontal cortex clusters reported in table 2 and 3 nor between in-scanner reports of post-reappraisal affect and activation magnitudes of both amygdala and prefrontal cortex clusters reported in table 2 and 3 in high risk individuals.

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Table 2

Table 3

Discussion

For many years researchers have strived to illuminate the pathophysiological mechanisms of MDD, however progress has been modest and definitive answers to the most pressing questions remain unclear. For instance, it is well documented that some people are more vulnerable to depression than the others. However, we still know relatively little about the neurobiological markers of depression vulnerability. Insight in this process will aid understanding into the mechanisms underlying the onset of MDD. A plausible way of clarifying the mechanisms underlying the onset of MDD is conducting research on individuals

who are at high risk for developing depression but have not yet experienced clinically significant symptoms.

We designed this study to extend the literature on the neurobiological markers of depression vulnerability using functional magnetic resonance imaging (fMRI) of cognitive reappraisal. Consistent with the literature, we predicted that the high risk group, relative to their low risk group, would report more difficulties in using reappraisal strategies and show greater activation in the amygdala in response to negative stimuli, and less activation in prefrontal cortex regions during repairing negative affect. In contrast to our expectations, both behavioural and neuroimaging findings suggest that high risk individuals are equally effective as low risk individuals in using cognitive reappraisal strategies. At first glance, this finding may seem counterintuitive given the rich neuroimaging literature demonstrating the aberrant functioning of amygdala and prefrontal cortex in emotional processing in high risk individuals. However, the discrepancy between our study and previous studies may be account for by several factors.

Firstly, as mentioned above behavioural results of this study showed that the two groups did not differ with respect to using effective reappraisal strategies to ameliorate negative affect. Interestingly, the same finding was also observed in remitted depressed patients (Ehring et al. , 2010, Smoski et al. , 2013). By implication, it would seem that depression vulnerability may not be linked to the effectiveness of cognitive reappraisal. Alternatively, difficulties in using cognitive reappraisal strategies may be confined to acute depression.

Secondly, it has been estimated that 45% of children with depressed mothers will develop a depressive episode by age 20 (Beardslee et al. , 1998) . Given the age range of our participants (18-24 years), our high risk group may include resilient participants instead of vulnerable participants, or a greater number of resilient participants than vulnerable participants. In addition, since MDD manifests over the whole lifespan, participants in low risk group are still at risk for developing depression. Therefore, we cannot exclude the

possibility that some of those participants will develop clinical depression in the future. Considered collectively, the lack of difference between groups may be explained by the equivocal probability of MDD manifestation in our sample.

Thirdly, the neurodevelopmental viewpoint may explain the discrepancy between our study and previous studies. Consistently, the studies reporting altered amygdala and prefrontal cortex activation were performed on high risk individuals in childhood and the adolescence period (Joormann, Cooney, 2012, Levesque, Beauregard, 2011, Monk, Klein, 2008). However, research in normative human development has shown that dramatic structural and functional changes emerge in both amygdala, prefrontal cortex, and amygdala-prefrontal cortex connections across childhood and adolescence (Gabard-Durnam et al. , 2014, Gee et al. , 2013b, Koolschijn and Crone, 2013, Sowell et al. , 2002, Toga et al. , 2006).

Healthy children and adolescents show dissimilar amygdala reactivity and amygdala-prefrontal cortex functional connectivity in response to emotional stimuli (Gabard-Durnam, Flannery, 2014). Typically, children display higher amygdala reactivity and immature functional connectivity (positive coupling) between the amygdala and prefrontal cortex in response to emotional stimuli, whereas adolescents and adults exhibit lower amygdala reactivity and mature functional connectivity (negative coupling) between amygdala and prefrontal cortex (Gee, Humphreys, 2013b). The shift from an immature amygdala-prefrontal coupling phenotype to a mature coupling phenotype occur during the transition from childhood to adolescence (Gabard-Durnam, Flannery, 2014). Interestingly, recent neuroimaging findings suggested that early adverse caregiving by virtue of maternal depression or deprivation may affect both the structural development of amygdala and the maturation timing of amygdala-PFC connectivity (Gee et al. , 2013a, Lupien et al. , 2011, Mehta et al. , 2009, Tottenham et al. , 2010).

For instance, in a seminal study, Lupien et al. followed offspring of women with recurrent depression and offspring of never-depressed women from birth to 10-year-old (Lupien,

Parent, 2011). At the end of this 10-year period, they found that offspring of depressed mothers had larger amygdala volume than the offspring of never-depressed mothers even though they haven't experienced a depressive episode. The same finding was also observed in children exposed to early maternal deprivation (Mehta, Golembo, 2009, Tottenham, Hare, 2010). Another seminal study by Gee et al. showed that previously institutionalized (PI) children (a model of early maternal deprivation) display early and increased reactivity of amygdala in response to negative emotional stimuli, which may in turn accelerate the development of amygdala-prefrontal cortex maturation (Gee, Gabard-Durnam, 2013a). Importantly, these functional alterations were mediated by increased cortisol levels. Although PI children as a group exhibit higher than average anxiety, further analyses examining individual differences within group revealed that PI participants with accelerated connectivity (negative connectivity, adult-like connectivity) had lower anxiety scores than the PI children with positive connectivity. It is also worth noting that anxiety scores were associated with amygdala-prefontal cortex connectivity, but not amygdala or prefrontal cortex reactivity. Based on these findings, Gee et al. interpreted that this accelerated maturation induced by early amygdala hyperactivity may be a developmental adaptation of the children to cope with the unexpected stressful conditions elicited by severely altered caregiving environment.

By implication, in contrast to previous claims, observed functional alterations in emotion regulation related brain structures of high risk individuals across childhood and adolescence may reflect atypical maturation of those brain structures rather than depression vulnerability. In addition, higher than average anxiety scores may instantiate early adverse caregiving environments and early maturation of amygdala-prefrontal connections. Consistent with this view, reported successful reappraisal along with heightened STAI-S and STAI-T scores by high risk individuals may arise from early maturation of amygdala-prefrontal connections.

Another finding that needs to be discussed is the heightened DERS-Clarity (Lack of Emotional Clarity) scores in our high risk group. The clarity subscale of DERS assesses

perceived emotional clarity, and is composed of five self-report items asking individuals to rate the way they experience their feelings (e.g. "I have no idea how I am feeling," " I have difficulties making sense out of my feelings," "I know exactly how I am feeling") (Gratz and Roemer, 2004). Higher scores on this subscale indicate greater levels of deficits in emotional clarity.

Emotional clarity (EC) is defined as the ability to understand, label, identify, and differentiate one's own emotional experiences (Coffey et al. , 2003, Gohm and Clore, 2002). In the present study, we found that unaffected high risk individuals (first degree relatives of depressed patients) are characterised by impaired (lower) perceived EC. To our knowledge our study is the first to show impaired perceived EC in first degree relatives of depressed patients. The same finding was also observed in both depressed patients (Loas et al. , 1998) and individuals in remission (Ehring et al. , 2008). Moreover, researchers have shown that deficits in emotional clarity can predict increases in depressive symptoms over time in children (Flynn and Rudolph, 2010) and may serve as vulnerability to depression in adolescents (Stange et al. , 2013). Based on these findings, we propose that low EC may be an endophenotype for MDD.

Our study has several limitations which should to be raised. Perhaps the greatest limitation of the study design is the lack of the assessment of pre-regulation affect for each stimulus. Therefore, we could not directly evaluate the group differences in subjective success of reappraisal. However, we found no group differences in self-reported negative affect to attend negative trials, suggesting that high and low risk groups did not differ significantly in terms of their emotional reactivity to negative stimuli.

The other limitation of this study is the relatively small sample size. This may lead to reduced statistical power to detect changes in whole-brain analyses and limit the generalisation of results to the general population. The lack of a psychometric tests that can directly evaluate the reappraisal in both groups may be considered another limitation. Lastly, since we didn't

record the exact time of maternal depression, we cannot definitively conclude that our participants display early maturation of the emotion regulation circuit.

Despite these limitations, this is the first study to examine neural correlates of reappraisal, and the first to show impaired perceived EC in high risk individuals.

Conclusion

In conclusion, in contrast to our expectations, results of the current study have shown no difference between high and low risk individuals in using cognitive reappraisal strategies and activation of brain structures implicated in cognitive reappraisal. Interestingly, our results suggest that high risk individuals are characterised by low perceived EC. Therefore, it might be interesting for future studies to explore whether high risk individuals differ from controls in the neural correlates of perceived EC which may help in clarifying the neural underpinnings of depression.

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Declaration of interest

The authors report no conflicts of interest.

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Table 1. Demographic and Clinical Characteristics of Participants

	RSK(N=16)	CTL(N=15)	p value
Age(years)	21.94 (2.32)	22.53 (1.80)	0.62
Education (years)	14.62 (2.44)	15.13 (1.72)	0.77
HDRS	2.86 (2.06)	1.53 (2.35)	0.08
STAI-S	39.21 (8.76)	30.53 (9.28)	0.02*
STAI-T	44.20 (10.02)	37.00 (9.41)	0.04*
DERS-TOTAL	83.70 (28.05)	68.80 (16.37)	0.17
DERS-Awareness	11.33 (3.47)	10.40 (2.52)	0.48
DERS-Clarity	11.26 (4.86)	8.13 (2.09)	0.03*
DERS-Strategies	18.53 (8.21)	12.86 (3.58)	0.06
DERS-Goals	16.20 (4.58)	15.33 (4.99)	0.62
DERS-Impulse	14.06 (6.65)	11.00 (3.79)	0.21
DERS-Nonacceptance	12.06 (4.60)	10.40 (3.92)	0.30

Standart Deviations are shown in parantheses.*$p<0.05$
CTL = control group; RSK = high-risk group; HDRS = Hamilton Depression Rating Scale; STAI-S = The Spielberger State -Trait Anxiety Inventory-State; STAI-T = The Spielberger State -Trait Anxiety Inventory-Trait; DERS = Difficulties in Emotion Regulation Scale

Table 2. Areas of Increased Activation in Response to Contrasts of Interest Within the RSK group

Contrast	Region	Side	BA	Ke	x	y	z	Z
View Neg > View Neutral								
	Amygdala	L		106	-16	-8	-14	5.73*
		R		85	18	-4	-16	5.30*
	Superior temporal gyrus	L		1154	-44	-56	16	6.38
	Middle temporal gyrus	R		1096	56	-66	14	6.13
	Medial frontal gyrus	L	9	553	-2	54	24	5.58
	Middle frontal gyrus	R	6	784	40	10	56	5.50
	Middle frontal gyrus	R	9	106	42	14	40	4.98*
	Middle frontal gyrus	L	46	60	-44	16	28	4.58*
	Inferior frontal gyrus	R		387	30	20	-18	5.96
	Lingual gyrus	L		1059	-2	-78	-12	6.84
	Fusiform gyrus	R		401	40	-48	-20	6.89
	Precuneus	R	7	72	6	-76	42	5.37
Reappraise Neg > Attend Negative								
	Superior frontal gyrus	L	9	241	-18	54	34	5.54*
	Middle frontal gyrus	L	9	52	-32	24	42	4.47*
	Superior frontal gyrus	R		969	16	60	26	6.11
	Superior frontal gyrus	L	8	267	-24	34	50	5.76
	Inferior frontal gyrus	L	47	19	-44	22	-12	5.01
	Medial frontal gyrus	L		18	-10	32	36	4.95
	Superior temporal gyrus	R	39	358	54	-62	26	5.82
	Precuneus	L	31	267	-16	-46	34	5.85
	Cuneus	L		551	-6	-92	10	6.25
	Caudate	L		40	-16	6	18	5.00
	Cerebellum	L		86	-32	-80	-28	5.62
	Cerebellum	R		160	14	-80	-36	5.18

R, Right; L, left; x, y, z, respective MNI coordinates of peak voxel activation; Z, Z value; all results $p < 0.05$ family-wise error corrected for multiple comparisons across whole brain; *p0.05 family-wise error corrected for anatomical a priori ROI.

Table 3. Areas of Increased Activation in Response to Contrasts of Interest Within the CTL group

Contrast	Region	Side	BA	Ke	x	y	z	Z
View Neg > View Neutral								
	Amygdala	L		132	-20	-8	-14	4.23 *
		R		155	18	-6	-14	4.81 *
	Superior temporal gyrus	R		171	44	16	-28	5.98
	Superior temporal gyrus	L		56	-32	12	-26	5.16
	Middle temporal gyrus	R		135	58	-4	-14	5.43
	Middle temporal gyrus	R		152	52	-68	18	5.37
	Middle frontal gyrus	L		71	46	22	26	5.21
	Middle frontal gyrus	R	46	60	48	20	26	5.04 *
	Middle frontal gyrus	L	46	8	-44	16	26	4.20 *
	Superior frontal gyrus	R	9	152	4	58	30	5.22 *
	Brainstem	R		350	6	-26	-2	5.76
Reappraise Neg > Attend Negative								
	Superior frontal gyrus	L	9	261	-2	56	36	5.66 *
	Superior frontal gyrus	R	9	115	-40	26	42	5.40 *
	Inferior frontal gyrus	L		169	-58	18	-6	6.12
	Middle frontal gyrus	L		127	-42	18	48	5.63
	Supramarginal gyrus	R		156	60	-54	36	6.00
	Superior temporal gyrus	R		126	46	18	-22	5.84
	Middle temporal gyrus	L		222	-44	-68	24	5.93
	Anterior cingulate	L	32	114	-8	44	12	5.59
	Precuneus	L	31	40	-4	-50	30	5.48
	Cerebellum	L		325	-32	-78	-32	6.04

R, Right; L, left; x, y, z, respective MNI coordinates of peak voxel activation; Z, Z value; all results $p < 0.05$ family-wise error corrected for multiple comparisons across whole brain; *p0.05 family-wise error corrected for anatomical a priori ROI.

FIGURE LEGENDS

Figure 1. Experimental design for a single trial. The experiment consisted of 96 trials with 48 neutral and 48 negative images. Each trial was composed of 5 events. First, a neutral/negative image was shown 4 second with the written instruction "View". Second, the image remained on the screen for an additional 4 second with an instruction either to "Reappraise" or "Attend". Third, a blank screen was shown for 4 second. Fourth, participants were instructed to rate their current emotion strenght. Finally, a word ''Relax'' appeared on the screen, indicating that participants should relax before the onset of the next trial. A single trial lasted 22 second.

Figure 2. Both high risk (RSK) and healty control (CTL) participants showed increased bilateral amygdala activation in response to negative emotional stimuli. ROI analyses on the left and corresponding group and condition averaged signal intensities for the left (L) and right (R) amygdala clusters are on the right. ($p<0.05$ for ROI). Error bars depict standard error of the mean(SEM).

Figure 1

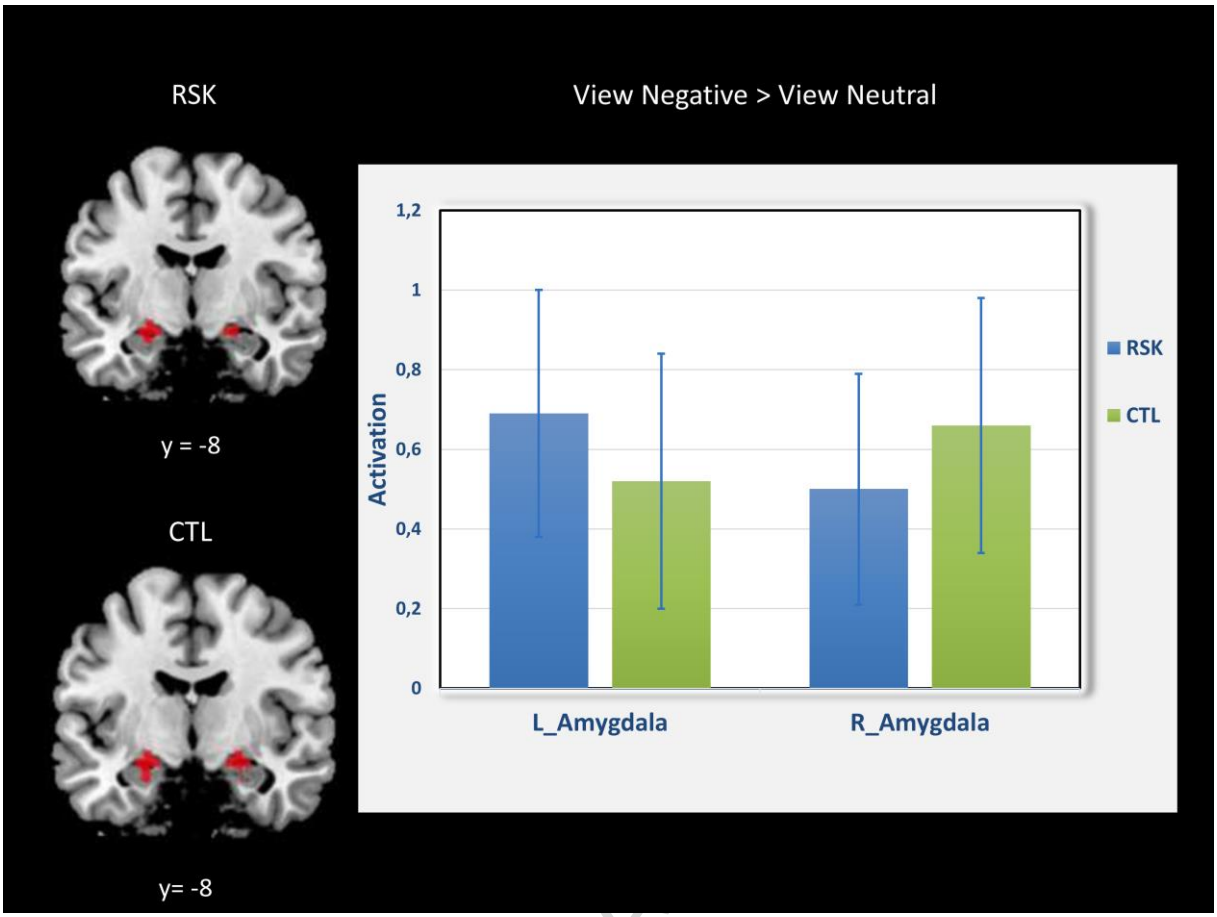

Figure 2